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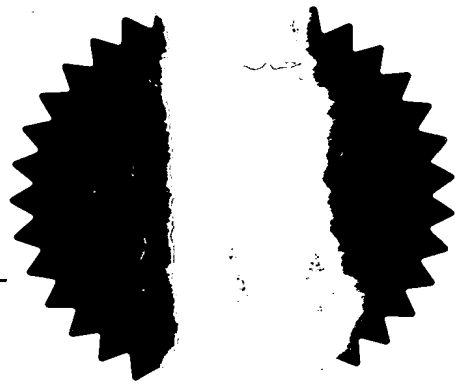

Signed Andrew Gerrey

Dated 17 December 1997

For Official use

28 JUN 1995

Our Reference: RJS/rsn/B45072

9513107.4

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Request for grant of a Patent

Form 1/77 **Patents Act 1977**

Rule 16 of the Patents Rules 1990 is the main rule governing the completion and filing of this form.

❶ Title of invention

1 Please give the title of the invention **VACCINES**

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15(4) (Divisional) ☐ 8(3) ☐ 12(6) ☐ 37(4) ☐

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If you are declaring priority from a PCT Application please enter 'PCT' as the country and enter the country code (for example, GB) as part of the application number

Please give the date in all number format, for example, 31/05/90 for 31 May 1990

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❼

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❽ *Please supply duplicates of claim(s), abstract, description and drawings).*

❽ Checklist

8a Please fill in the number of sheets for each of the following types of document contained in this application

Continuation sheets for this Patents Form 1/77 ☐

Claim(s)	2	Description	14
Abstract	--	Drawing(s)	--

8b Which of the following documents also accompanies the application?

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Priority documents *(please state how many)* ☐

Translation(s) of Priority documents *(please state how many)* ☐

Patents Form 7/77 - Statement of Inventorship and Right to Grant ☐

Patents Form 9/77 - Preliminary Examination Report ☐

Patents Form 10/77 - Request for Substantive Examination ☐

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Vaccines

The present invention relates to novel vaccine formulations, to methods of their production and to their use in medicine. In particular, the present invention relates to vaccines containing an antigen, an immunologically active fraction derived from the bark of Quillaja Saponaria Molina such as QS21, and a sterol.

Immunologically active saponin fractions having adjuvant activity derived from the bark of the South American tree Quillaja Saponaria Molina are known in the art. For example QS21, also known as QA21, an Hplc purified fraction from the Quillaja Saponaria Molina tree and its method of its production is disclosed (as QA21) in US patent No. 5,057,540. Quillaia saponin has also been disclosed as an adjuvant by Scott et al, Int. Archs. Allergy Appl. Immun., 1985, 77, 409. However, the use of QS21 as an adjuvant is associated with certain disadvantages. For example when QS21 is injected into a mammal as a free molecule it has been observed that necrosis, that is to say, localised tissue death, occurs at the injection site.

It has now surprisingly been found that necrosis at the injection site can be avoided by use of formulations containing a combination of QS21 and a sterol. Preferred sterols include β-sitosterol, stigmasterol, ergosterol, ergocalciferol and cholesterol. These sterols are well known in the art, for example cholesterol is disclosed in the Merck Index, 11th Edn., page 341, as a naturally occuring sterol found in animal fat.

In a first aspect the present invention therefore provides a vaccine composition comprising an antigen, an immunologically active saponin fraction and a sterol. Preferably the compositions of the invention contain the immunologically active saponin fraction in substantially pure form. Preferably the compositions of the invention contain QS21 in substantially pure form, that is to say, the QS21 is at least 90% pure, preferably at least 95% pure and most preferably at least 98% pure. Other immunologically active saponin fractions useful in compositions of the invention include QA17/QS17. Compositions of the invention comprising QS21 and cholesterol show decreased reactogenicity when compared to compositions in which the cholesterol is absent. In addition it is known that QS21 degrades under basic conditions where the pH is about 7 or greater. A further advantage of the present compositions is that the stability of QS21 to base-mediated hydrolysis is enhanced in formulations containing cholesterol.

Preferred compositions of the invention are those forming a liposome structure. Compositions where the sterol/immunologically active saponin fraction forms an ISCOM structure also form an aspect of the invention.

The ratio of QS21 : sterol will typically be in the order of 1 : 100 to 1 : 1 weight to weight. Preferably excess sterol is present, the ratio of QS21 : sterol being at least 1 : 2 w/w. Typically for human administration QS21 and sterol will be present in a vaccine in the range of about 1 μg to about 100 μg, preferably about 10 μg to about 50 μg per dose.

The liposomes preferably contain a neutral lipid, for example phosphatidylcholine, which is preferably non-crystalline at room temperature, for example eggyolk phosphatidylcholine, dioleoyl phosphatidylcholine or dilauryl phosphatidylcholine. Preferably the liposomes also contain a charged lipid which increases the stability of the liposome structure. Preferably the amount of charged lipid is about 1-20% w/w, most preferably 5-10%. The ratio of sterol to phospholipid is 1-50% (mol/mol), most preferably 20-25%.

Preferably the compositions of the invention contain MPL (3-deacylated mono-phosphoryl lipid A, also known as 3D-MPL). 3D-MPL is known from GB 2 220 211 (Ribi) as a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem, Montana. A preferred form is disclosed in International Patent Application 92/116556.

Preferred compositions of the invention are those wherein liposomes are initially prepared without MPL, and MPL is then added, preferably as 100 nm particles. The MPL is therefore not contained within the vesicle membrane (known as MPL out).

Often the vaccines of the invention will not require any specific carrier and be formulated in an aqueous or other pharmaceutically acceptable buffer. In some cases it may be advantageous that the vaccines of the present invention will further contain alum or be presented in an oil in water emulsion, or other suitable vehicle, such as for example, liposomes, microspheres or encapsulated antigen particles.

Preferably the vaccine formulations will contain an antigen or antigenic composition capable of eliciting an immune response against a human or animal pathogen. Antigen or antigenic compositions known in the art can be used in the compositions of the invention, including antigen or antigenic compositions derived from HIV-1, (such as

gp120 or gp160), any of Feline Immunodeficiency virus, human or animal herpes viruses, such as gD or derivatives thereof or Immediate Early protein such as ICP27 from HSV1 or HSV2, cytomegalovirus (especially human)(such as gB or derivatives thereof), Varicella Zoster Virus (such as gpI, II or III), or from a hepatitis virus such as hepatitis B virus for example Hepatitis B Surface antigen or a derivative thereof, hepatitis A virus, hepatitis C virus and hepatitis E virus, or from other viral pathogens, such as Respiratory Syncytial virus (for example HSRV F and G proteins or immunogenic fragments thereof disclosed in US Patent 5149650 or chimeric polypeptides containing immunogenic fragments from HSRV proteins F and G, eg FG glycoprotein disclosed in US Patent 5194595), human papilloma virus or Influenza virus, or derived from bacterial pathogens such as Salmonella, Neisseria, Borrelia (for example OspA or OspB or derivatives thereof), or Chlamydia, or Bordetella for example P.69, PT and FHA, or derived from parasites such as plasmodium or toxoplasma.

HSV Glycoprotein D (gD) or derivatives thereof is a preferred vaccine antigen. It is located on the viral membrane, and is also found in the cytoplasm of infected cells (Eisenberg R.J. et al; J of Virol 1980 35 428-435). It comprises 393 amino acids including a signal peptide and has a molecular weight of approximately 60 kD. Of all the HSV envelope glycoproteins this is probably the best characterised (Cohen et al J. Virology 60 157-166). In vivo it is known to play a central role in viral attachment to cell membranes. Moreover, glycoprotein D has been shown to be able to elicit neutralising antibodies in vivo and protect animals from lethal challenge.

A truncated form of the gD molecule is devoid of the C terminal anchor region and can be produced in mammalian cells as a soluble protein which is exported into the cell culture supernatant. Such soluble forms of gD are preferred. The production of truncated forms of gD is described in EP 0 139 417. Preferably the gD is derived from HSV -2.

An embodiment of the invention is a truncated HSV-2 glycoprotein D of 308 amino acids which comprises amino acids 1 through 306 naturally occuring glycoprotein with the addition Asparagine and Glutamine at the C terminal end of the truncated protein devoid of its membrane anchor region. This form of the protein includes the signal peptide which is cleaved to allow for the mature soluble 283 amino acid protein to be secreted from a host cell.

In another aspect of the invention Hepatitis B surface antigen is a preferred vaccine antigen.

As used herein the expression 'Hepatitis B surface antigen' or 'HBsAg' includes any HBsAg antigen or fragment thereof displaying the antigenicity of HBV surface antigen. It will be understood that in addition to the 226 amino acid sequence of the HBsAg antigen (see Tiollais et al, Nature, 317, 489 (1985) and references therein) HBsAg as herein described may, if desired, contain all or part of a pre-S sequence as described in the above references and in EP-A- 0 278 940. In particular the HBsAg may comprise a polypeptide comprising an amino acid sequence comprising residues 12-52 followed by residues 133-145 followed by residues 175-400 of the L-protein of HBsAg relative to the open reading frame on a Hepatitis B virus of ad serotype (this polypeptide is referred to as L*; see EP 0 414 374). HBsAg within the scope of the invention may also include the pre-S1-preS2-S polypeptide described in EP 0 198 474 (Endotronics) or close analogues thereof such as those described in EP 0 304 578 (Mc Cormick and Jones). HBsAg as herein described can also refer to mutants, for example the 'escape mutant' described in WO 91/14703 or European Patent Application Number 0 511 855A1, especially HBsAg wherein the amino acid substitution at position 145 is to arginine from glycine.

Normally the HBsAg will be in particle form. The particles may comprise for example S protein alone or may be composite particles, for example (L*,S) where L* is as defined above and S denotes the S-protein of HBsAg. The said particle is advantageously in the form in which it is expressed in yeast.

The preparation of hepatitis B surface antigen S-protein is well documented. See for example, Harford et al (1983) in Develop. Biol. Standard 54, page 125, Gregg et al (1987) in Biotechnology, 5, page 479, EP 0 226 846, EP 0 299 108 and references therein.

•The formulations within the scope of the invention may also contain an anti-tumour antigen and be useful for immunotherapeutically treating cancers.

Vaccine preparation is generally described in New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978. Encapsulation within liposomes is described, for example, by Fullerton, U.S. Patent 4,235,877. Conjugation of proteins to macromolecules is disclosed, for

example, by Likhite, U.S. Patent 4,372,945 and by Armor et al., U.S. Patent 4,474,757.

The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Generally, it is expected that each dose will comprise 1-1000 mcg of protein, preferably 2-100 mcg, most preferably 4-40 mcg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. Following an initial vaccination, subjects may receive one or several booster immunisation adequately spaced.

The formulations of the present invention maybe used for both prophylatic and therapeutic purposes.

Accordingly in a further aspect, the invention provides a method of treatment comprising administering an effective amount of a vaccine of the present invention to a patient.

The following examples and data illustrates the invention.

Examples

1.1 Method of preparation of liposomes:

A mixture of lipid (such as phosphatidylcholine either from egg-yolk or synthetic) and cholesterol in organic solvent, is dried down under vacuum (or alternatively under a stream of inert gas). An aqueous solution (such as phosphate buffered saline) is then added, and the vessel agitated until all the lipid is in suspension. This suspension is then extruded under high pressure through a polycarbonate membrane with a pore-size of 0.1 µm and finally sterile filtered through a 0.2 µm filter. The extrusion through polycarbonate membranes is achieved using a commercial thermobarrel extruder. Microfluidization or sonication could replace this step.
Typically the cholesterol:phosphatidylcholine ratio is 1:4 (w/w), and the aqueous solution is added to give a final cholesterol concentration of 5 mg/ml. If MPL in organic solution is added to the lipid in organic solution the final liposomes contain MPL in the membrane (referred to as MPL in).
The liposomes have a defined size of 100 nm and are referred to as SUV (for small unilamelar vesicles). If this solution is repeatedly frozen and thawed the vesicles fuse to form large multilamellar structures (MLV) of size ranging from 500nm to 15 µm.
The liposomes by themselves are stable over time and have no fusogenic capacity.

1.2 Formulation procedure:

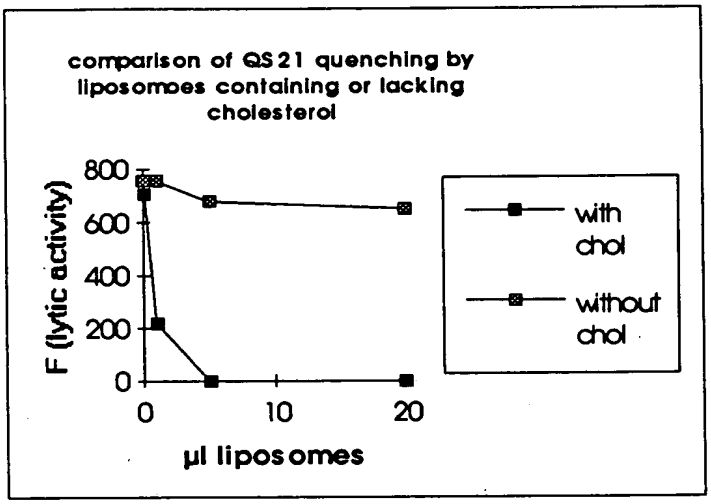
QS21 in aqueous solution is added to the liposomes. This mixture is then added to the antigen solution which may if desired contain MPL in the form of 100nm particles.

1.3 The lytic activity of QS21 can be inhibited by liposomes containing cholesterol.

When QS21 is added to erythrocytes, they lyse them releasing hemoglobin. This lytic activity can also be measured using liposomes which contain cholesterol in their membrane and an entrapped fluorescent dye, carboxyfluorescein: as the liposomes are lysed the dye is released which can be monitored by fluorescence spectroscopy. If the fluorescent liposomes do not contain cholesterol in their membrane no lysis of the liposomes is observed.

If the QS21 is incubated with liposomes containing cholesterol prior to adding it to erythrocytes, the lysis of the erythrocytes is diminished depending on the ratio of cholesterol to QS21. If a 1:1 ratio is used no lytic activity is detected. If the liposomes do not contain cholesterol, inhibition of lysis requires a one thousand fold excess of phospholipid over QS21.

The same holds true using fluorescent liposomes to measure the lytic activity.
In the graph below the lytic activity of 4 µg of QS21 treated with liposomes lacking cholesterol (1 mg eggyolk lecithin per ml) or containing cholesterol (1 mg lecithin, 500 µg cholesterol per ml) was measured by fluorescence.

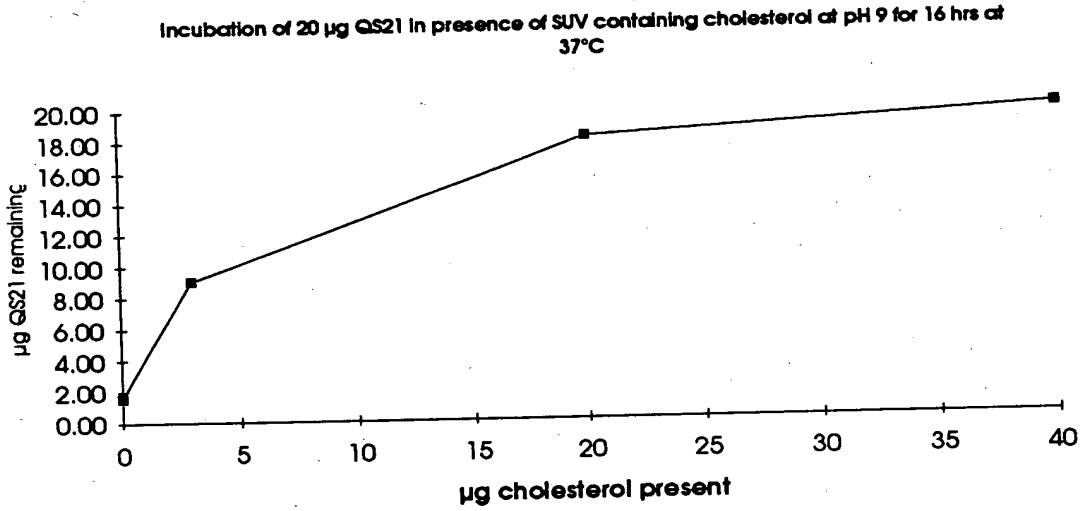


The data shows that QS21 associates in a specific manner with cholesterol in a membrane, thus causing lysis (of cells or fluorescent liposomes).
If the QS21 first associates with cholesterol in liposomes it is no longer lytic towards cells or other liposomes. This requires a minimum ratio of 0.5:1 chol:QS21(w/w).

Cholesterol is insoluble in aqueous solutions and does not form a stable suspension. In the presence of phospholipids the cholesterol resides within the phospholipid bilayer which can form a stable suspension of vesicles called liposomes. To avoid the requirement to add phospholipids a soluble derivative was tried. Polyoxyethanyl-cholesterol sebacate is soluble in water at 60 mg/ml however even at a 2000 fold excess (w/w) over QS21 no reduction in the lytic activity of QS21 was detected.

1.4 Increased stability of QS21 by liposomes containing cholesterol.

QS21 is very susceptible to hydrolysis at a pH above 7. This hydrolysis can be monitored by measuring the decrease in the peak corresponding to QS21 on reverse-phase HPLC. For example, the graph below shows that at pH 9 and at a temperature of 37°C, 90% of QS21 is hydrolysed within 16 hours. If liposomes containing cholesterol are added to the QS21 at a ratio of 2:1 (chol:QS21 w/w) no hydrolysis of the QS21 is detected under the same conditions. If the ratio is 1:1 10% of the QS21 is degraded.



It is concluded that when QS21 associates with liposomes containing cholesterol it becomes much less susceptible to base-mediated hydrolysis.

The hydrolysis product is described as having no adjuvant activity when given parenterally, hence vaccines containing QS21 have to be formulated at acidic pH and kept at 4°C to maintain adjuvant composition. The use of liposomes may overcome this requirement.

1.5 Reactogenicity studies:

Mice injected in tibialis muscle with 5 µg QS21 (or escin or digitonin) added to increasing quantities of liposomes (expressed in terms of µg cholesterol). Lytic activity is expressed as µg QS21 equivalent, which means that quantity of QS21 required to achieve the same hemolysis as the sample.

Redness, necrosis and toxicity in the muscle at the site of injection were scored visually after sacrificing the mice.

formulation	lytic activity	redness	necrosis	toxicity
QS21 +PBS	5	+++	±	+++
QS21 +1 µg chol (SUV)	4	+++	+	++++
QS21 +5 µg chol (SUV)	0	-	-	±
QS21+25 µg chol (SUV	0	±	-	+
SUV alone	0	-	-	-
digitonin	5	-	-	±
PBS	0	-	-	-

The data shows that when the lytic activity is abolished by the addition of liposomes containing cholesterol the toxicity due to the QS21 is also abolished.

1.6 Reactogenicity intra-muscularly in rabbits

Values in U.I./L

Experiment	Formulation	JO	hemolysis	J1	hemolysis	J3	hemolysis
Rabbit n°1		1078	±	8650		1523	
Rabbit n°2		1116		4648		1435	
Rabbit n°3	**QS21 50µg**	660		4819		684	
Rabbit n°4		592		5662		684	
Rabbit n°5		3400		7528		1736	
Mean		**1369**		**6261**		**1212**	
SD		**1160**		**1757**		**495**	

Experiment	Formulation	JO	hemolysis	J1	hemolysis	J3	hemolysis
Rabbit n°6		596		1670		460	
Rabbit n°7		540		602		594	
Rabbit n°8	QS21 50µg	611		1873		803	
Rabbit n°9	Chol in SUV 50µg	521		507		616	
Rabbit n°10	(1:1)	1092	±	787		555	
Mean		**672**		**1088**		**606**	
SD		**238**		**636**		**125**	

Experiment	Formulation	JO	hemolysis	J1	hemolysis	J3	hemolysis
Rabbit n°11		332		344		387	
Rabbit n°12		831		662		694	
Rabbit n°13	QS21 50µg	464		356		519	
Rabbit n°14	Chol in SUV 150µg	528		720		614	
Rabbit n°15	(1:3)	1027		568		849	
Mean		**637**		**530**		**613**	
SD		**285**		**173**		**175**	

Experiment	Formulation	JO	hemolysis	J1	hemolysis	J3	hemolysis
Rabbit n°16		540		769		745	
Rabbit n°17		498		404		471	
Rabbit n°18	QS21 50µg	442		717		(4535)	
Rabbit n°19	Chol in SUV 250µg	822		801		925	
Rabbit n°20	(1:5)	3182	±	2410		960	
Mean		**1097**		**1020**		**775**	(1527)
SD		**1175**		**793**		**224**	(1692)

Experiment	Formulation	JO	hemolysis	J1	hemolysis	J3	hemolysis
Rabbit n°21		321		290		378	
Rabbit n°22		660		535		755	
Rabbit n°23	PBS	650		603		473	
Rabbit n°24		1395		(3545)		(5749)	
Rabbit n°25		429	±	323		263	
Mean		**691**		**438**	(1059)	**467**	(1523)
SD		**419**		**155**	(1396)	**210**	(2369)

The data shows that the addition of cholesterol-containing liposomes to the formulation significantly reduces the elevation in CPK (creatine phospho kinase) caused by the QS21. Since the CPK increase is a measure of muscle damage this indicates decreased muscle damage and is confirmed by the histopathology.

1.7 Binding of the liposome-QS21 complex to alum.

QS21 was incubated with neutral liposomes containing excess cholesterol as well as radioactive cholesterol and then incubated with alum ($Al(OH)_3$) in PBS. Alone, neither neutral liposomes nor QS21 bind to alum in PBS, yet negatively charged liposomes do. When together however, QS21 and neutral liposomes bind to alum. The supernatant contained neither QS21 (assayed by orcinol test) nor radioactive cholesterol.
This suggests that the QS21 has bound to the liposomes and permits the liposome-QS21 combination to bind to the alum. This may arise from a negative charge being imposed on the liposomes by the QS21, or to an exposure of hydrophobic regions on the liposomes. The results also imply that QS21 does not extract cholesterol from the membrane.
This suggests that compositions of the invention can be used in alum based vaccines.

1.8 Comparaison of liposomal QS21/MPL and free QS21+MPL for antibody and CMI induction

SUV prepared by extrusion (EYPC:chol:MPL 20:5:1).
For MPL out, liposomes prepared without MPL and MPL added as 100 nm.
QS21 added prior to antigen. Chol:QS21 = 5:1 (w/w)

MLV made by freeze-thawing SUV 3x prior to antigen addition.
To have antigen entrapped: antigen added to SUV prior to freeze-thawing and QS21 added after freeze-thaw. Antigen encapsulation =5% in, 95% out.
-mice (balb/c for gD, B10BR for RTSs) injected twice footpad.

ag = 10 µg **RTSs**	anti HBsAg Titres		
formulation	IgG1	IgG2a	IgG2b
SUV/QS + MPL(out) + Ag	1175	10114	71753
SUV/QS/MPL(in) + Ag	-------	-------	-------
MLV/QS + MPL(out) + Ag	2247	11170	41755
MLV/QS/MPL(in) + Ag	969	7073	18827
MLV/QS/MPL(in)/Ag(in) + Ag	1812	2853	9393
QS + MPL + Ag	372	9294	44457
Ag	<100	<100	<100
SUV/QS + MPL(out)	<100	<100	<100
MLV/QS/MPL(in)	<100	<100	<100

ag = 20µg **gD**	anti-gD	CMI
formulation	IgG	IL2 48hr pg/ml
SUV/QS + MPL(out) + Ag	2347	960
SUV/QS/MPL(in) + Ag	2036	15
MLV/QS + MPL(out) + Ag	1578	15
MLV/QS/MPL(in) + Ag	676	15
MLV/QS/MPL(in)/Ag(in) + Ag	1064	15
QS + MPL + Ag	1177	15
Ag	<100	44
SUV/QS + MPL(out)	<100	15
MLV/QS/MPL(in)	<100	105

The data shows that SUV/QS+MPL(out) induces high antibody titres at least as good as QS21+MPL, as well as inducing IL2 a marker of cell mediated immunity, while quenching QS21 reactogenicity.

Additional results from a second experiment comparing QS21 and quenched QS21 in mice with HSV gD as antigen are shown below.

				Isotypes 7 post II					
Formulation	antigen	IgG 7 post II (GMT)	IgG 14post II (GMT)	IgG1 µg/ml	%	IgG2a µg/ml	%	IgG2b µg/ml	%
SUV/QS21 + MPL out	gD (5µg)	20290	16343	331	26	716	56	222	17
SUV/QS21/MPLin	gD (5µg)	12566	10731	418	44	412	44	111	12
QS21+MPL	gD (5µg)	10504	10168	200	34	285	48	107	18
SUV/QS21 + MPL out	none	0	0	0	0	0	0	0	0
QS21	gD (5µg)	3468	4132	156	66	67	28	14	6
SUV/QS21	gD (5µg)	11253	11589	484	57	304	36	65	8

1.9 Comparison of gp120 plus liposomal MPL/QS21 with free MPL/QS21

Lipos = SUV containing MPL in the membrane

Chol:QS = 6:1

Response tested two weeks after one immunisation

formulation	**proliftn**	**IFN-g** ng/ml	**IL2** pg/ml	**IL5** pg/ml
lipo/MPL/QS21 + Ag	12606	16.6	59	476
MPL+QS21+Ag	16726	15.8	60	404

After second immunisation:

formulation	**proliftn**	**IFN-g** ng/ml	**IL4** pg/ml	**IL5** pg/ml
lipo/MPL/QS21 + Ag	12606	135	0	250
MPL+QS21+Ag	16726	60	0	500

The data shows that QS21 quenched in liposomes containing MPL induces Th1/Th0 response equal to MPL+QS21.

At this ratio of cholesterol to QS21, QS21 is non-toxic in rabbits (by CPK).

1.10 Comparaison of QS21 with 0,1 and 5 fold excess of cholesterol as adjuvant in HSV prophylactic model

Model = guinea pigs injected with gD2t combined with 50 or 100µg QS21 with a 1:1 or 5:1 excess of cholesterol in the form of SUVs.

added to gD	Elisa 28 days post II	Protection %
50 chol:50 QS	35000	100
100 chol 100 QS	24000	50
250 chol 50 QS	8000	64
50 QS	38000	50

2. Therapeutic Vaccines

2.1 Formulation of HBsAg L*,S particles.

HBsAg L*,S particles may be formulated as follows:

10µg of HBsAg L*,S particles/dose are incubated 1h. at room temperature under agitation. The volume is adjusted using water for injection and a PBS solution and completed to a final volume of 70µl/ dose with an aqueous solution of QS21 (10µg/dose). pH is kept at 7 ± 0.5.

Similar formulations may be prepared using 1 and 50µg of HBsAg L*,S and also using the HBsAg S antigen.

These formulations may be tested in the Woodchuck surrogate therapeutic model using Woodchuck HBV antigens as a model.

Woodchuck model

DQ (i.e. quenched) QS21 may be tested in the woodchuck therapeutic model were animals are chronically infected with the virus. Specific woodchuck hepatitis virus vaccine may be add mixed with QS21 as such or DQ and with or without MPL and administered to the animals every months for 6 months.
Effectiveness of the vaccine may be assess through viral DNA clearance.

2.2 Guinea Pig Model (HSV)

Formulations containing gD in combination with QS21 (DQ or such) and MPL may be used in therapy. A suitable model using guinea pigs is described in European patent application No. 0576 478. Briefly, guinea pigs infected with HSV-2 strain MS are vaccinated with the vaccine of the invention and followed for evidence of recurrent disease.

Claims

1. A vaccine composition comprising comprising an antigen, an immunologically active saponin fraction and a sterol.

2. A vaccine composition according to claim 1 wherein the immunologically active saponin fraction is QS21.

3. A vaccine composition according to claim 1 or 2 wherein the sterol is cholesterol.

4. A vaccine composition according to claim 2 or 3 wherein the ratio of QS21:sterol is from 1:100 to 1:1.

5. A vaccine composition according to any of claims 1 to 4 which further contains MPL.

6. A vaccine composition as claimed herein comprising an antigen or antigenic composition derived from any of Human Immunodeficiency Virus, Feline Immunodeficiency Virus, Herpes Simplex Virus type 1, Herpes Simplex virus type 2, Human cytomegalovirus, Hepatitis A, B, C or E, Respiratory Syncytial virus, human papilloma virus, Influenza virus, Salmonella, Neisseria, Borrelia, Chlamydia, Bordetella, Plasmodium or Toxoplasma.

7. A vaccine as claimed in any of claim 1 to 5 wherein the antigen is a tumour antigen.

8. Use of composition as defined in any of claims 1 to 5 for the manufacture of a vaccine for the prophylatic treatment of viral, bacterial, or parasitic infections.

9. Use of composition as defined in any of claims 1 to 5 for the manufacture of a vaccine for the immunotherapeutic treatment of viral, bacterial, parasitic infections or cancer.

10. A method of treating a mammal suffering from or susceptible to a pathogenic infection comprising the administration of a safe and effective amount of a composition according to any of claims 1 to 5.

11. A method of treating a mammal suffering from cancer comprising the administration of a safe and effective amount of a composition according to any of claims 1 to 5.

12. A process for making a vaccine composition according to claim 1 comprising admixing an immunologically active saponin fraction and cholesterol with an antigen or antigenic composition.